

Factors Affecting the Reversed-Phase Liquid Chromatographic Separation of Polycyclic Aromatic Hydrocarbon Isomers¹⁾

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Reversed-phase
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Summary

Reversed-phase liquid chromatography (LC) on C₁₈ stationary phases provides excellent selectivity for the separation of polycyclic aromatic hydrocarbons (PAH). Recent studies have shown that several factors affect selectivity for the LC separation of PAH including phase type (monomeric or polymeric), pore diameter and surface area of the silica substrate, and surface density of the C₁₈ ligands. In this paper the separation of eleven PAH isomers of molecular weight 278 is used to further illustrate the effect of stationary phase characteristics and shape of the solute (length-to-breadth ratio, L/B) on retention and selectivity. Only polymeric C₁₈ phases with a high C₁₈ surface coverage provided separation of all eleven isomers and the elution order of these isomers generally followed increasing L/B values. The effect of solute nonplanarity on reversed-phase LC retention was investigated on both monomeric and polymeric phases using a series of planar and nonplanar PAH pairs. For each solute pair, the nonplanar solute eluted earlier than the planar solute, the largest selectivity factors being observed on the C₁₈ phase with the highest percent carbon load. Based on these studies, a model is proposed to describe the retention of PAH on polymeric C₁₈ phases.

1 Introduction

The complex mixtures of polycyclic aromatic hydrocarbons (PAH) encountered in environmental samples contain numerous isomeric structures. Since certain isomers are often more mutagenic and/or carcinogenic than other isomers, the separation and quantification of individual isomeric PAH are necessary to assess accurately the potential health effects of exposure to various pollutant sources. Even using high efficiency open tubular gas

chromatography (GC), a number of isomeric PAH are still difficult to separate on conventional nonpolar stationary phases, e.g., chrysene and triphenylene; benzo[b]fluoranthene, benzo[j]fluoranthene, and benzo[k]fluoranthene; dibenz[a,c]anthracene and dibenz[a,h]anthracene [1].

Recently, reversed phase liquid chromatography (LC) on chemically-bonded C₁₈ stationary phases has been shown to provide excellent selectivity for the separation of PAH isomers including methyl-substituted PAH isomers [2]. Several studies [3-6] have shown that C₁₈ columns from various manufacturers provide different retention and selectivity characteristics for PAH. Recent studies have determined that several factors affect the selectivity of reversed-phase C₁₈ phases for PAH separations, including phase type (monomeric or polymeric) [7], pore diameter and surface area of the silica substrate [8], and surface coverage or C₁₈ ligand density [7,9]. In general, these studies found that polymeric C₁₈ phases, which are prepared using trichlorosilane reagents with the addition of a small amount of water, provide greater selectivity for the separation of PAH isomers than do the more common and widely used monomeric phases, which are prepared using monochlorosilane reagents with the exclusion of water. In addition, the greatest selectivity is achieved for polymeric C₁₈ phases prepared on wide pore (>150 Å diameter) silica substrates with low surface area (<100 m²/g) [7,8].

In addition to the investigations of the influence of stationary phase parameters on retention and selectivity in reversed-phase LC, other studies have examined the correlation of reversed-phase retention data for PAH with various molecular structure descriptors. Wise *et al.* [2] described a relationship between the shape of PAH solutes, defined as the length-to-breadth ratio (L/B), and reversed-phase LC retention. Hasan and Jurs [10] used this

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LC data set [2] to predict retention based on numerical descriptors from the PAH molecular structures (e.g., molecular connectivity, substructure environment, and geometric and calculated property descriptors). Jino and Kawasaki [11] correlated reversed-phase LC retention for 26 PAH on several different stationary phases with five descriptors: a topological descriptor (molecular connectivity, χ), a geometric descriptor (van der Waals volume, V_w), a calculated physical property descriptor (logarithm of partition coefficient in *n*-octanol/water), a correlation factor (*F*) as defined by Hurtubise *et al.* [12], and the shape parameter (*L/B*). They found that all of these descriptors, except *L/B*, showed high correlation with LC retention. This is not surprising since all of these descriptors, except *L/B*, are related to the molecular weight of the PAH. However, these parameters provide little or no differentiation among isomeric PAH. The length-to-breadth ratio, on the other hand, does differentiate between isomers since it provides a measure of the shape of the PAH, but it has little meaning when comparing non-isomeric PAH, as in the study of Jino and Kawasaki [11]. In the study of Wise *et al.* [2], molecular connectivity and *L/B* were combined in a correlation with retention to account for differences in molecular weight and to differentiate among isomers. Earlier studies [2,7] also showed that the planarity of the PAH greatly affects the retention and selectivity on C_{18} stationary phases.

In this paper we report further investigation of the factors affecting selectivity in the separation of PAH isomers. In particular, the separation of eleven PAH isomers of molecular weight 278 was used to illustrate the effect of stationary phase characteristics and of the shape (*L/B*) of the solute on retention and selectivity. The effect of PAH nonplanarity on retention was further investigated on monomeric and polymeric phases. As a result of these studies, a model describing the retention of PAH on polymeric C_{18} phases is proposed.

2 Experimental*

Materials. The PAH isomers of molecular weight 278 were obtained from the following sources: benzo[*b*]chrysene and benzo[*c*]chrysene (Community Bureau of Reference, Belgium); dibenz[*a,j*]anthracene (National Cancer Institute Chemical Carcinogen Repository, Bethesda, MD); dibenz[*a,c*]anthracene and dibenz[*a,h*]anthracene (Fluka Chemical Corp., Hauppauge, NY); dibenzo[*c,g*]phenanthrene (J. F. McKay, Laramie Energy Technology Research Center, Laramie WY); benzo[*a*]naphthacene, pentaphene,

and dibenzo[*b,g*]phenanthrene (W. Schmidt, Biochemical Institute of Environmental Carcinogens, Ahrensburg, FRG); benzo[*g*]chrysene (R. G. Harvey, University of Chicago, Chicago, IL); picene (Pfaltz and Bauer, Inc., Stamford, CT). The additional standards used for the comparison of nonplanar and planar solutes were obtained from the following sources: benzo[*ghi*]perylene (Community Bureau of Reference), coronene and *p*-quaterphenyl (Aldrich Chemical Co., Inc., Milwaukee, WI); benzo[*c*]phenanthrene and phenanthro[3,4-*c*]phenanthrene (Alfred Bader Library of Rare Chemicals, Aldrich Chemical Co., Inc.), *m*-tetraphenyl (ICN Pharmaceuticals, Inc., K & K Laboratories, Plainville, NY); 1,3,5-triphenylbenzene (Fluka Chemical Corp.), benzo[*ghi*]fluoranthene (R. Lao, Environmental Protection Programs Directorate, Ottawa, Canada).

Columns. Commercial columns and columns prepared in our laboratory were used to study PAH retention. Separations of the mixture of PAH isomers of molecular weight 278 were performed on 5 μ m particle size Vydac 201 TP columns used in a previous study and designated as lot numbers 10, 12, 14, 15, and 16 [9]. Lot number 12 is representative of phase loadings normally provided by the manufacturer, lot 14 has a slightly higher loading, but is available upon request from the manufacturer. Columns from lots 15 and 16 were specially prepared by the manufacturer to have higher than normal phase loading and lot 10 was prepared to have lower than normal phase loading. The phase loading values for these columns listed in the text were calculated as described previously [7] assuming an average specific surface area of 84 m²/g for the unbounded silica. In the previous study [9] the phase loading values were calculated using surface areas for the bonded substrates (thus the two types of surface coverage values differ). Monomeric and polymeric C_{18} phases were also synthesized in our laboratory using a previously published procedure [7]. Polymeric phases were synthesized on 5 μ m Vydac TP silica (The Separations Group, Hesperia, CA; 84 m²/g surface area, 330 Å pore diameter), and on 10 μ m Polygosil silica (Macherey Nagel, Duren, FRG; 245 m²/g surface area, 150 Å pore diameter). The monomeric phases were prepared on the 10 μ m Polygosil silica. For the polymeric phases, 4 g of dry silica was added to 100 mL CCl_4 containing 10 mL of octadecyltrichlorosilane. Low, normal and heavy load polymeric phases were synthesized using 0.2 mL, 0.5 mL and 1.5 mL of water, respectively. The reaction slurries were refluxed for 15 h. The monomeric phase was prepared by adding 7.8 g of silica to 100 mL CCl_4 containing 20 g dimethyloctadecylchlorosilane and 2 mL pyridine. Percent carbon values for these bonded phases were determined as described previously [13].

Chromatography. A liquid chromatograph consisting of two reciprocating piston pumps, solvent programmer, 20 μ L loop injector, and 254 nm fixed wavelength detector was used in the retention studies. The PAH isomers of molecular

* Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

weight 278 were separated using a linear gradient of 85 to 100% acetonitrile in water over 15 minutes. Each of the planar/nonplanar PAH probes were chromatographed individually at 85% acetonitrile in water and 100% acetonitrile.

3 Results and Discussion

Recent studies [7,8] have shown that greater selectivity in the reversed-phase LC separation of PAH is achieved on polymeric C₁₈ phases prepared on wide pore (300 Å) silica substrates than on the more commonly used monomeric phases prepared on narrow pore silica (60-100 Å). In these studies the separation of the 16 PAH on the US Environmental Protection Agency's priority pollutant list was compared on monomeric and polymeric C₁₈ phases prepared on a variety of silica substrates. The 16-component PAH

mixture contains several pairs of isomers, i.e., phenanthrene/anthracene, pyrene/fluoranthene, benz[a]anthracene/chrysene, benzo[b]fluoranthene/benz[k]fluoranthene/benzo[a]pyrene, and benzo[ghi]perylene/indeno[1,2,3-cd]pyrene. The two isomers pairs benz[a]anthracene/chrysene and benzo[ghi]perylene/indeno[1,2,3-cd]pyrene are generally the most difficult to separate on monomeric C₁₈ phases, but they can be separated easily on polymeric C₁₈ phases. Larger groups of PAH isomers, i.e., three to four isomers per group, were used in an earlier study on the effect of PAH shape on the reversed-phase LC retention [2]. In general, isomers were found to elute in the order of increasing L/B values. Reversed-phase LC elution orders for several larger isomer sets consisting of methyl-substituted PAH were also in good agreement with the calculated L/B values.

The largest isomeric group of non-substituted PAH investigated in the previous study [2] contained five isomers of molecular weight 278, i.e., dibenz[a,j]anthracene, dibenz[a,c]anthracene, dibenz[a,h]anthracene, benzo[b]chrysene, and picene. Since there are twelve possible cata-condensed PAH isomers of this molecular weight, we selected this group of isomers to verify further the correlation of PAH shape (L/B) with retention and to investigate the selectivity of reversed-phase LC for isomer separations. The structures of these twelve isomers and their length-to-breadth ratios (L/B), calculated as described previously [2], are shown in **Figure 1**.

The separation of these isomers was first evaluated on a C₁₈ polymeric phase with a high C₁₈ surface coverage (loading) since previous work [9] had indicated that such phases had better selectivity for PAH isomer separations. The separation of 11 of the 12 possible isomers in Figure 1 is shown in **Figure 2**. The remaining isomer, pentacene, was not included due to limited solubility in the mobile phase. However, pentacene does elute significantly later than picene on a polymeric C₁₈ phase as would be expected from the large L/B value [14]. In general, the elution order follows increasing L/B values. One isomer with an apparent anomalous behavior is dibenz[a,c]anthracene (L/B = 1.24). However, it might also be argued that benzo[g]chrysene (L/B = 1.32) and dibenz[b,g]phenanthrene (L/B = 1.33), which contain the nonplanar benzo[c]phenanthrene structure, are eluting earlier than expected due to the solute nonplanarity (see discussion to follow). This same argument would explain the separation of benzo[c]chrysene and dibenz[a,j]anthracene which both have L/B values of 1.47. A similar elution order for the GC separation of all twelve of these isomers has been obtained on a liquid crystal phase [15]; however, a conventional nonpolar GC stationary phase (i.e. SE-54) was unsuccessful in separating all of these isomers. Benzo[a]naphthacene (L/B = 1.77) has greater retention than would be predicted by the L/B





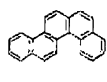
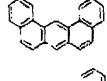
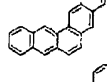
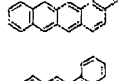
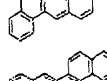
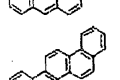
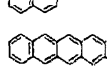
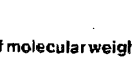
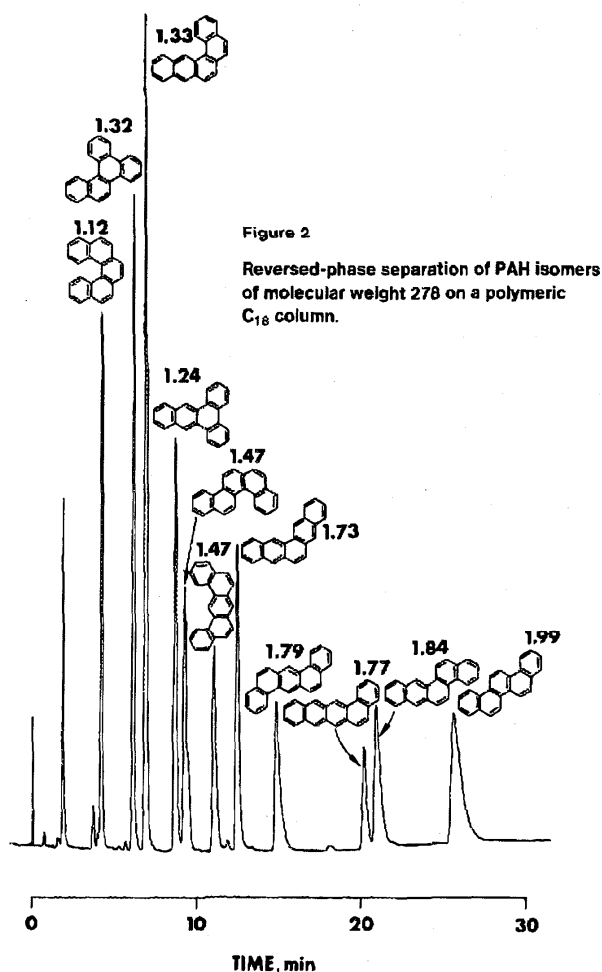
PEAK ID	STRUCTURE	IDENTIFICATION	L/B
1)		Dibenz[cd]phenanthrene	1.12
4)		Dibenz[a,c]anthracene	1.24
2)		Benzo[g]chrysene	1.32
3)		Dibenz[b,g]phenanthrene	1.33
5)		Benzo[c]chrysene	1.47
6)		Dibenz[a,j]anthracene	1.47
7)		Pentaphene	1.73
9)		Benzo[a]naphthacene	1.77
8)		Dibenz[a,h]anthracene	1.79
10)		Benzo[b]chrysene	1.84
11)		Picene	1.99
12)		Pentacene	2.22

Figure 1

PAH isomers of molecular weight 278 and their corresponding length-to-breadth ratios.



value. This may indicate that the naphthalene structure (*i.e.*, four rings with linear annellation) has greater influence on the retention than the overall L/B value of the PAH

The effect of C₁₈ surface coverage (loading) on the separation of these isomers is shown in **Figure 3**. The isomer mixture was separated on four polymeric C₁₈ columns with increasing C₁₈ surface coverage *i.e.*, column A (low load, 2.7 $\mu\text{mol}/\text{m}^2$) to column D (high load, 5.1 $\mu\text{mol}/\text{m}^2$). All of the isomers were resolved only on the high load column (D). The chromatogram in Figure 2 was also obtained on a column with a similar high C₁₈ surface coverage (5.9 $\mu\text{mol}/\text{m}^2$). The elution order of isomers 9 and 10 in Figure 3 (benzo[a]naphthalene and benzo[b]chrysene), changed from the low to high surface coverage columns. Similar behavior (*i.e.*, elution order reversal) has been observed for the four-ring cata-condensed isomers benz[a]anthracene and chrysene. This observation is of particular interest since benzo[a]naphthalene and benzo[b]chrysene contain the same structural features as benz[a]anthracene and chrysene, respectively.

The separations shown in Figures 2 and 3 were obtained on commercial polymeric C₁₈ phases prepared on wide pore silica (330 Å pore diameter). Polymeric C₁₈ phases prepared in our laboratory on 330 Å pore diameter silica (columns

Figure 3

Reversed-phase separation of PAH isomers on four polymeric C₁₈ columns (A-D) with increasing C₁₈ loading (See Figure 1 for peak identifications).

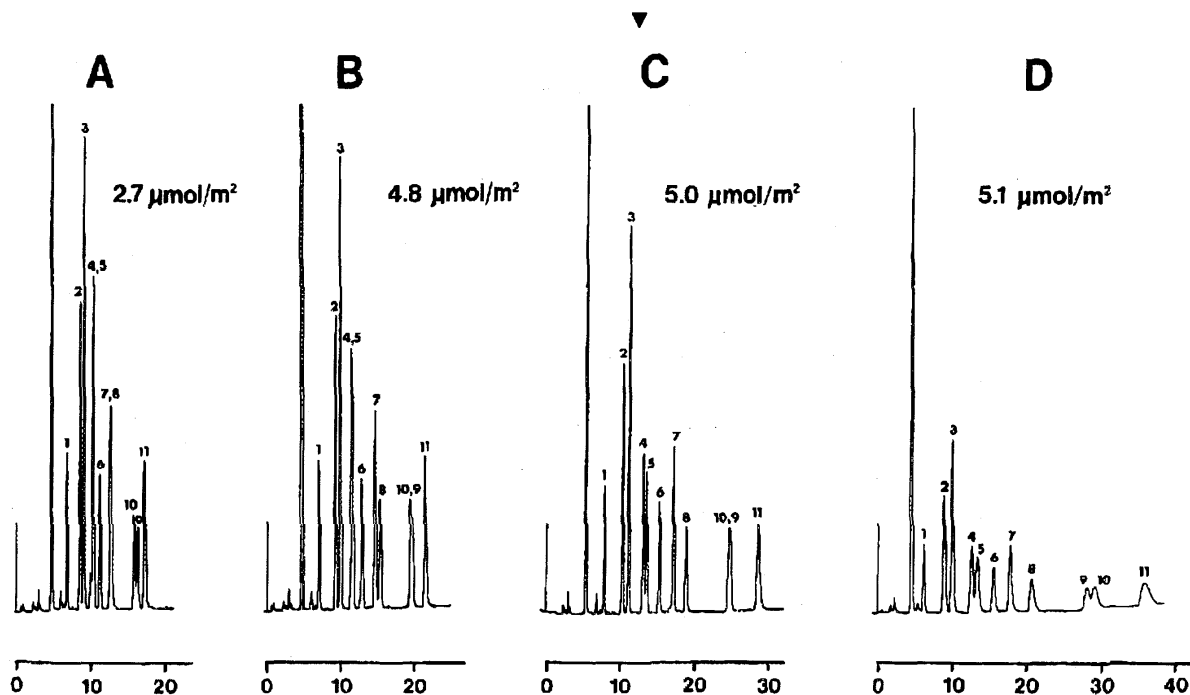


Table 1
Percent carbon, surface area, and C_{18} surface coverage values for C_{18} phases synthesized in this study.

No	C_{18} Phase	% Carbon	Surface area	Surface coverage ^{a)} ($\mu\text{mol}/\text{m}^2$)
1	Monomeric	12.4	245	2.52
2	Polymeric	17.4	245	4.50
3	Monomeric	14.4	245	3.01
4	Polymeric (low)	5.87	84	3.55
5	Polymeric (normal)	7.50	84	4.67
6	Polymeric (high)	7.84	84	4.91

a) Calculated as described in ref. 7

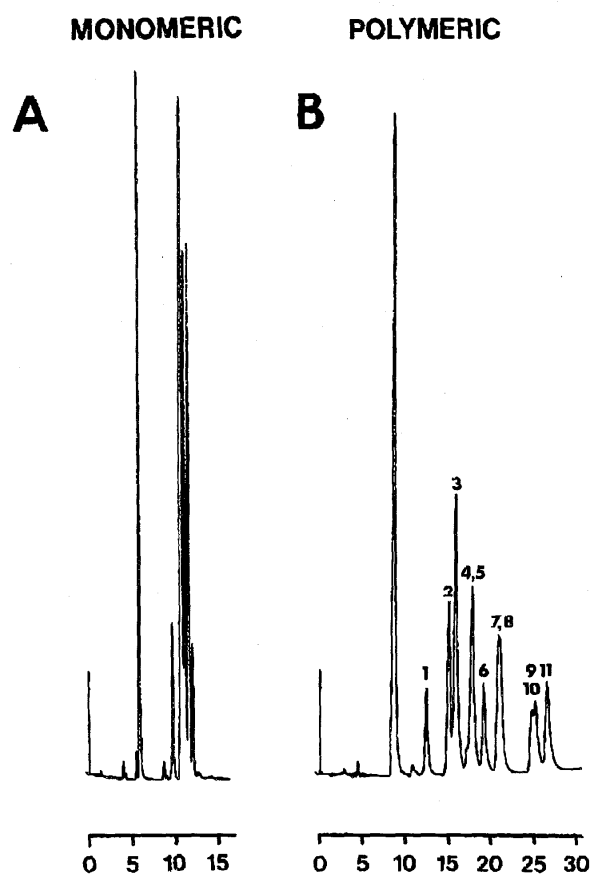


Figure 4

Comparison of monomeric and polymeric C_{18} phases (columns 1 and 2 in Table 1) for the separation of PAH isomers (see Figure 1 for peak identifications). Gradient: 80-100% acetonitrile in water over 20 minutes.

4-6 in Table 1) provided similar selectivities for the separation of these isomers. In Figure 4 the separation of these isomers on monomeric and polymeric phases, which were prepared on a silica substrate with 150 Å pore diameter, are

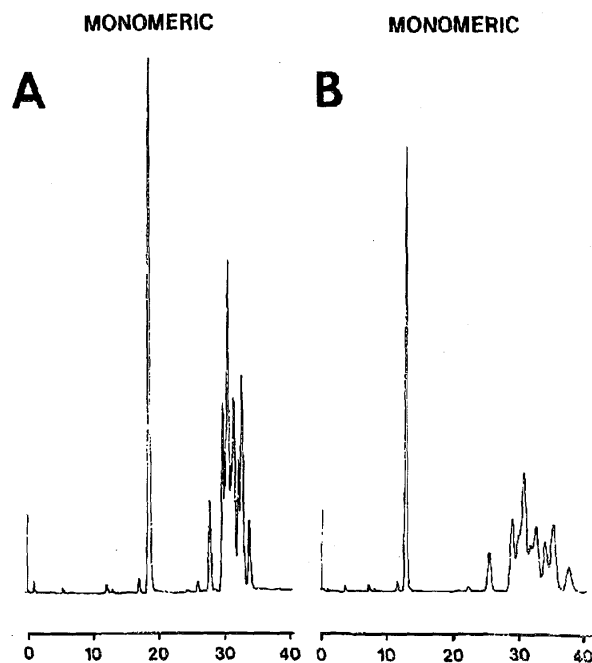


Figure 5

Separation of PAH isomers on a monomeric C_{18} phase (Column 1 in Table 1). A) 60-80% acetonitrile in water over 20 minutes. B) Isocratic at 70% acetonitrile in water.

compared. As shown in Table 1, the percent carbon loadings for these two phases are 12.4% ($2.52 \mu\text{mol}/\text{m}^2$) for the monomeric phase and 17.4% ($4.50 \mu\text{mol}/\text{m}^2$) for the polymeric phase. Since the monomeric phase had lower absolute retention, a weaker solvent gradient (Figure 5A) and isocratic conditions at 70% acetonitrile in water (Figure 5B) were used in an attempt to improve resolution on the monomeric phase. However, as illustrated in Figures 4 and 5, the polymeric phase provides much greater selectivity than the monomeric phase for the isomer separation. Comparison of Figure 4B with Figure 3A indicates that the polymeric phase on the 150 Å pore diameter silica provides a selectivity similar to the low load polymeric phase on the wide pore silica. Polymeric C_{18} phases prepared on narrow pore silica (60-100 Å diameter) have less selectivity for the separation of PAH than phases prepared on wide pore silica as shown in a previous study on the effect of pore size on selectivity [8].

Previous studies [2,7,9] have indicated that nonplanar PAH have significantly different behavior on monomeric vs polymeric C_{18} phases. Based on these observations, Sander and Wise [7] used a three-component mixture of planar and nonplanar PAH to evaluate the selectivity characteristics of C_{18} phases. A series of planar and nonplanar PAH were used in this study to investigate further the effect of nonplanarity on solute retention. Pairs of PAH solutes

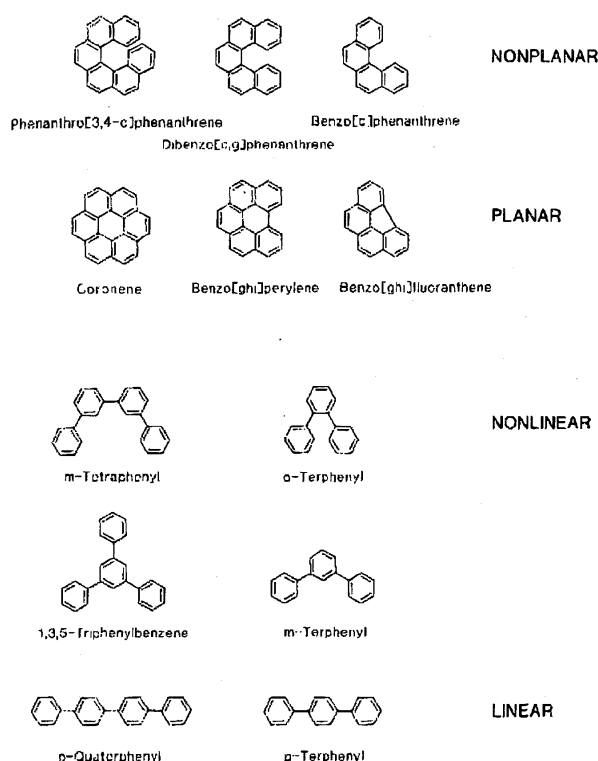


Figure 6

Structures of planar/nonplanar and linear/nonlinear PAH pairs.

(see Figure 6) were selected with similar overall shape and length-to-breadth ratios, but with different degrees of planarity. For example, phenanthro[3,4-c]phenanthrene

has a helical conformation due to opposing hydrogen atoms at the terminal aromatic rings. In contrast, coronene is completely planar. Similarly, dibenzo[c,g]phenanthrene and benzo[c]phenanthrene are nonplanar, while benzo[ghi]perylene and benzo[ghi]fluoranthene are planar. Linear and nonlinear solutes were also studied (Figure 6), including positional isomers of terphenyl and tetraphenyl (quaterphenyl).

Four columns were used in these retention studies. Low, moderate (normal), and high load polymeric C_{18} phases were synthesized on a wide pore silica substrate and a monomeric C_{18} phase was synthesized on a narrow pore silica. The percent carbon loadings and the surface coverage values for these four columns (numbers 3-6) are summarized in Table 1.

Selectivity factors for these solute pairs (k' ratio of planar/nonplanar or linear/nonlinear solutes) at 85% acetonitrile in water and 100% acetonitrile are summarized in Table 2. Several trends are apparent. In each instance, retention of the planar PAH was greater than their nonplanar analogs. Also, linear isomers of terphenyl and tetraphenyl were retained longer than *ortho* or *meta* isomers. Selectivity factors for all PAH pairs increased with increasing polymeric character of the bonded phase. This suggests that polymeric phases are more sensitive to differences in PAH planarity than are monomeric phases. In general, polymeric C_{18} phases are more selective towards PAH than monomeric phases, and generally better separations of PAH result with polymeric phases. Selectivity coefficients are also slightly greater at 100% than at 85% acetonitrile/water with the greatest differences observed for large values of K' . The difference between planar and nonplanar PAH retention further increases with the degree of nonplanarity.

Table 2

Planar/nonplanar selectivity coefficients^{a)}.

Compound	85% acetonitrile/water				100% acetonitrile			
	monomeric	low	polymeric normal	heavy	monomeric	low	polymeric normal	heavy
p/m-terphenyl	1.2	1.3	2.1	3.0	1.2	1.3	2.3	3.5
p/o-terphenyl	1.3	1.7	2.6	3.8	1.4	1.5	2.9	4.3
p/m-tetraphenyl	1.4				1.4	4.4	11.7	34.5
p-quaterphenyl/ 1,3,5-triphenylbenzene	1.5				1.5	3.0	14.2	46.0
benzo[ghi]fluoranthene/ benzo[c]phenanthrene	1.1	1.2	1.4	1.6	1.2	1.4	1.7	1.8
benzo[ghi]perylene/ dibenzo[c,g]phenanthrene	1.8	2.8	4.5	4.1	2.1	3.3	7.8	7.5
coronene/ phenanthro[3,4-c]phenanthrene	2.7	5.1	9.6		3.4	6.2	12.1	17.1

^{a)} Selectivity coefficient = k' ratio of planar/nonplanar or linear/nonlinear solutes

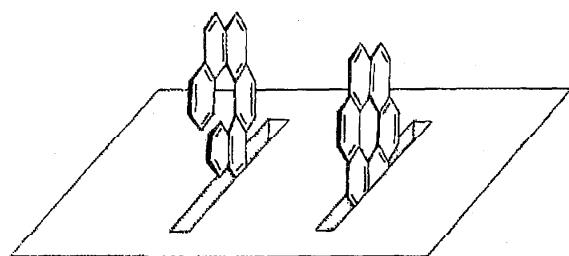


Figure 7

Schematic representation of the "slot model" for retention of PAH on polymeric C_{18} stationary phases.

Selectivity factors increase on all phases with increasing ring number of the solute pairs. Since the steric hindrance of opposing hydrogens also increases with ring number, [e.g., phenanthro[3,4-c]phenanthrene (6 rings) greater than dibenzo[c,g]phenanthrene (5 rings)], this correlates with the nonplanar character. Among the linear and nonlinear PAH, the selectivity factors (k' ratio of linear/nonlinear solutes) increased with nonlinearity. For example, on each phase the selectivity factors for *para/ortho* isomers of terphenyl were greater than those for the *para/meta* terphenyl isomers. Again, the largest differences were observed for the polymeric phases.

4 Retention Model

The retention behavior of the PAH studied can be explained in terms of a schematic representation of the bonded phase which we will refer to as the "slot model". In each of the cases examined, planar and linear molecules were preferentially retained over their nonplanar or nonlinear analogs. The planar and linear PAH interact more strongly with the bonded phases than the nonplanar and nonlinear PAH. A model can be envisaged in which the bonded phase consists of a number of narrow "slots" into which the solute molecules can penetrate (see Figure 7). Planar molecules would be able to fit more easily into these narrow slots and interact strongly with the C_{18} stationary phase, whereas the nonplanar molecules would not penetrate as far into the slots, and so would interact less strongly with the stationary phase. Since the planar/nonplanar PAH pairs have similar molecular weights and L/B values, the differences in retention are attributed to the increased "thickness" of the nonplanar solute which would hinder penetration of the solute into the narrow slots. If the model comprises both narrow and wide slots, then the correlation of retention behavior with the length-to-breadth ratio can also be explained. Wise et al. [2] have shown that among PAH isomers retention is greatest for the long, narrow molecules. Such molecules would fit into more of the available slots than would "square" molecules, and so would be

retained longer than square molecules. Viewed in this way, the property of nonplanarity is simply an extension of the molecular descriptor of length-to-breadth ratio to include a thickness parameter. The retention of linear and nonlinear molecules would follow a similar mechanism based on long, narrow solutes (more linear) having the greatest retention.

The relationship between phase selectivity and solute size and bonded phase length has been examined by a number of workers [16-19]. Lochmüller and Wiider [16] observed differences in retention behavior between large and small PAH. They attributed these differences to simultaneous changes in thermodynamic and phase ratio parameters that occur as a function of PAH size. Tchaplá and coworkers [17] found discontinuities in the retention of homologs, also as a function of size. They proposed that the effect was due to penetration of the solute into a finite stationary phase thickness. As solute size approached the chain length of the stationary phase, retention leveled off.

Martire and Boehm [20] in their "unified theory of retention and selectivity in liquid chromatography" concluded that chemically bonded phases exhibit shape selectivity which increases as the chains become more fully extended and that rigid-rod solutes have greater retention than globular solutes. It is clear that phase thickness or phase density (or both) increase with loading for the polymeric C_{18} phases. The fact that polymeric phases exhibit a greater selectivity for molecular shape than monomeric phases and that shape selectivity increases with increasing loading for polymeric phases suggests that polymeric phases may be more "extended" or more rigid than the monomeric phases. In addition, the shape selectivity observed for polymeric C_{18} phases is similar to that observed for liquid crystalline phases used in gas chromatography [15]. This similarity would also suggest that polymeric phases are more "ordered" than monomeric phases.

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